HOMEOSTASIS OF TAURINE AND GLUTAMATE PLASMA LEVELS AFTER ACUTE AND CHRONIC ETHANOL ADMINISTRATION IN MAN

Roberta J Ward[1*], Elizabeth J Marshall[2], David Ball[2], José Martinez[2] and Philippe De Witte[1]

[1]Biologie du Comportement, Université Catholique de Louvain, 1348 Louvain-la-Neuve, Belgium,

[2] Addiction Science Centre, Institute of Psychiatry, London UK.

(Accepted November 26, 1998)

SUMMARY

Taurine homeostasis is perturbed after acute and chronic ethanol administration. An oral dose of ethanol, (0.8 g / kg) significantly decreased the plasma taurine content 90 minutes after its administration, while glutamic acid content increased significantly at 30 minutes. Chronic alcohol abusers, showed significant increases in plasma taurine content 7 days after alcohol withdrawal. In 50% of these patients the taurine levels exceeding the reference range and correlated with the elevated levels of plasma glutamic acid. In addition in these patients there was also a significant decrease in anxiety score, CIWA Ar, ($p<0.01$). Although the correlation between taurine and glutamate has been described in other diseases this is the first time that such changes have been reported after acute or chronic ethanol administration.

Key words. taurine; alcohol abusers; alcohol detoxification; glutamic acid; amino acids

INTRODUCTION

Taurine is a sulphonated amino acid which is present in high concentrations, mM range, in many tissues (1). Its biological functions is possibly related to its influence upon calcium homeostasis, i.e. calcium transport, calcium binding and function, as well as being implicated in the activity of the membrane enzyme, phospholipid N-methyl transferase (2).

There are high concentrations of taurine in the brain of man, its concentration is comparable to GABA but lower than glutamate, where it plays many important functions. The structural similarity of the taurine transporter to neurotransmitter transporters is consistent with a neuromodulatory role for taurine in the nervous system (3). Brain glutamate and taurine concentrations correlate suggesting co-localisation (4) which has led to the suggestion that it may modulate the toxicity of this excitatory amino acid. The high phospholipid brain content may necessitate the presence of taurine for its methylation (5), while taurine also has important membrane stabilising actions by controlling the fluxes of both calcium and Cl' fluxes across membranes (1). Drugs such as ethanol which have dramatic and devastating effects on membrane

composition and structure (6), alter phospholipid metabolism (7) and increase glutamate release and concentration, particularly during detoxification, (8, 9), may indirectly effect taurine homeostasis.

There have been no studies of the effect of acute or chronic ethanol administration on taurine homeostasis in man. Acute ethanol administration evokes increases in the microdialysate taurine content of several rat brain regions (10, 11) due to a variety of factors which include osmoregulatory responses and changes in membrane fluidity (12). Certain brain regions e.g. hippocampus, show elevations in the basal taurine concentration at the cessation of four weeks of chronic alcoholisation in rats (8). Taurine supplementation prior to ethanol injection in mice will diminish alcohol-induced sleep-time (13) while homotaurine supplementation decreases craving in chronic alcohol misusers after alcohol detoxification (14). One study of six randomly selected chronic abusers of alcohol indicated that the circulating concentrations of taurine were slightly lower than normal (15).

However, as yet, there have been no investigations of taurine homeostasis after either acute or chronic ethanol administration in man which could yield some insight into the role of taurine during ethanol intoxication and withdrawal. Therefore in these present studies the circulating concentrations of taurine and other amino acids has been evaluated in normal subjects after an acute dose of ethanol and in chronic abusers of ethanol, while consuming high amounts of ethanol and then seven days after medically assisted withdrawal.

MATERIAL AND METHODS

Four fasting normal individuals, (daily consumption <1 unit) ingested an oral dose of alcohol, 0.8 g/kg, in the form of whisky, at 08.30. Blood specimens were taken prior to the ingestion of alcohol and then at 30, 60 and 90 minutes.

Blood specimens were collected from 22 Caucasian patients, 16 males, 6 females, admitted to the National Alcohol Inpatient Unit, at the Royal Bethlum Hospital, Beckenham, UK. All patients fulfilled ICD-10 / DSM IV criteria for alcohol dependence. On admission, each patient had a comprehensive physical, psychiatric and neuropsychological assessment. Extensive demographic, clinical and family history were collected and laboratory investigations undertaken. Alcohol withdrawal was assessed with the revised Clinical Institute Withdrawal Assessment, CIWA.Ar (16). The severity of dependence and level of alcohol problem were assessed by the Severity of Alcohol Dependent Questionnaire, SADQ (17), and Alcohol Problems Questionnaire, APQ, (18, 19), respectively. Comorbid psychiatric diagnoses (ICD-10 / DSM IV) were made by the consultant psychiatrist following clinical interview. Random urine toxicological screens were performed repeatedly during their admission to confirm drug-free status.

A blood specimen was taken at the time of planned admission, for the assay of alcohol levels, biochemical parameters and haematological indices. Heparinised blood specimens were taken, both at the time of admission and 7 days after detoxification, for the analysis of taurine and plasma amino acid. Blood samples were also taken from a further 20 controls, who were of comparable age to the patients, who consumed < 1 unit /day, for the analysis of taurine and plasma amino acids.

(Ethical permission for this study had been obtained from the Bethlum & Maudesley NHS Trust Research Ethical Committee),

Plasma amino acids, together with taurine, were analysed by an automated HPLC technique (Pharmacia Biotech biochrome 20) system, with UV detection after reaction with ninhydrin reagent.

Data analysis: The plasma amino acid results are presented as mean $\pm$ standard deviation. Differences between the time points after acute ethanol ingestion and between admission and detoxification in chronic

alcohol abusers were assessed by ANOVA design with Fischer's LSD (Protected t test). A GB statistical programme calculated correlations. All clinical analyses utilised the Statistical Package for the Social Sciences (SPSS) for Windows. Statistical significance was established by Chi test for categorical data; Fisher's exact test when the sample was insufficient to calculate chi-squared and a two-tailed student t test for comparison of means with interval data. Significance for all statistical analyses was set at less than 0.05.

RESULTS

Acute ethanol dose to normal subjects

The plasma ethanol concentration had increased at 30 minutes to a mean concentration of 129 mg% after the ingestion of ethanol. After 90 minutes the plasma alcohol concentration had marginally decreased by 15%. The plasma concentration of taurine decreased in each subject after acute ethanol ingestion, the mean concentration being significantly lower at 90 minutes by comparison to the basal level. Figure 1a. Alanine, a β-amino acid had decreased significantly at 30 minutes, $P<0.05$, Figure 1b, and continued to be significantly lower, $P<0.01$ at both 60 and 90 minutes. Glutamic acid increased significantly, $P<0.05$, at 30 minutes, after which time the levels decreased slowly to reach the basal level by 90 minutes, Figure 1c. All of the other amino acids assayed, neutral, basic, aromatic and branched chain, showed no significant alteration after acute ethanol ingestion.

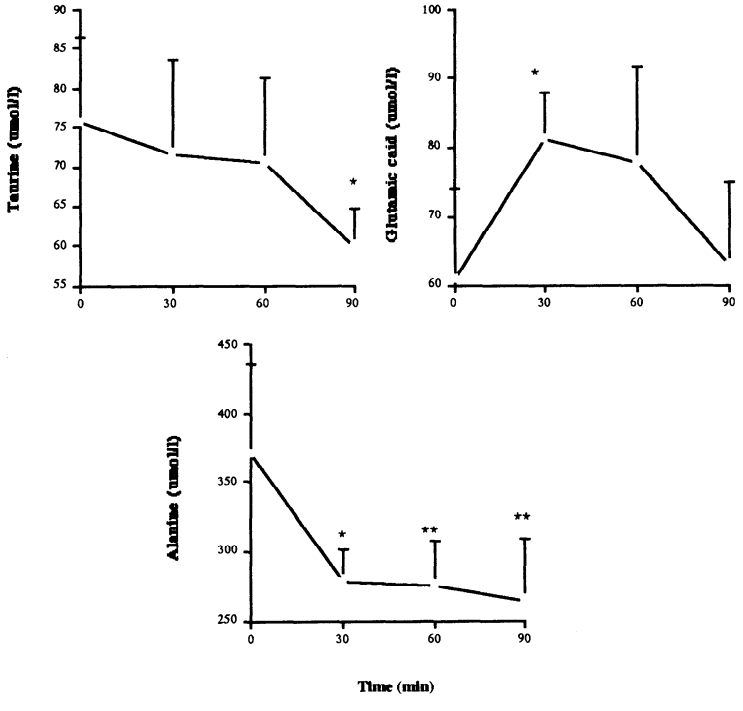

Figure 1. Changes in plasma concentrations of taurine (1a) alanine (1b) and glutamate (1c) after oral dose of ethanol (0.8 g/kg) to fasting male controls.

Chronic ethanol abuse.

On admission to the detoxification unit, 16 of the 22 subjects had detectable blood ethanol, range 2 - 434 mg%. In these patients the mean concentration of the amino acid glutamic acid was significantly raised, 151 ± 84 μmol/l (by comparison to the control subjects, 66 ± 25 μmol/l), and significantly correlated with the elevated activity of the liver enzyme γ glutamyl transferase, r= 0.67 P<0.001. All of the other mean concentrations of amino acids plus taurine, although significantly lower than those of the control subjects, nevertheless were within their appropriate reference ranges, Table 1.

Table 1. Concentrations of plasma amino acids and taurine in chronic alcohol abusers on admission to the Alcohol detoxification Unit and 7 days after detoxification, and control subjects.

		ALCOHOL ABUSERS n=22		CONTROLS n=20
	Amino Acid	Admission	After 7days detoxification	
Reference range				
27-100	Taurine	69.8 ± 14	92 ± 40	56 ± 9
10-85	Cystine	18.6±16	18.9± 12	22 ±13
6-40	Methionine	21.4± 8	22.4± 6	29 ± 9
35-90	Phenylalanine	51.1± 12	56.9 ± 12	62 ± 15
30-90	Tyrosine	57.4 ± 16	77.5 ± 17	75 ± 18
30-100	Histidine	70.9 ± 14	79.4 ± 14	75 ± 1
75-175	Leucine	87.6 ± 25*	100.9 ± 26	139 ± 39
35-100	Isoleucine	46.0 ± 18	54.2 ± 18	75 ± 23
100-335	Proline	205 ± 66**	201.8 ± 61$$	342 ± 109
70-195	Serine	86.1 ± 31	114.3 ± 27	108 ± 25
140-320	Valine	163.5 ± 35**	174.6 ± 42$$	250 ± 54
120-550	Glycine	184.0 ± 41*	236.8 ± 73§§	237 ± 56
210-650	Alanine	299.1 ± 90**	377 ± 72§§$$	476 ± 103
80-240	Lysine	130.7 ± 36**	168.0 ± 45§	198 ± 47
20-140	Arginine	66.2 ± 22	74.9 ± 26	105 ± 39
80-195	Threonine	120.9 ± 48	125.4 ± 40	136 ± 28
10-67	Glutamic Acid	151.1 ± 84**	140.5 ± 86$$	66 ± 25
1-25	Aspartic acid	9.2 ± 3.3	10.3 ± 3.3	11 ± 3
520-740	Glutamine	403 ± 131**	456.9 ± 180§§$$	544 ± 72

Significance calculated by ANOVA Fischer unpaired t test

Admission sample V Control*	** P<0.01	* P<0.05
Admission sample v 7 day sample §	§§ P<0.01	§ P<0.05
Control v 7 day sample $	$$ P<0.01	$ P<0.05

Seven days after admission the mean concentration of taurine had significantly increased, P<0.03, in the 22 patients, the plasma level of taurine exceeding its quoted reference range (27-100 μmol/l) in seven patients. Glutamic acid content also increased in 50% of patients, while each of the other plasma

amino acids showed an elevation in their concentration in each of the patients but remained within their appropriate reference ranges, Table 1. There was a significant correlation between taurine and glutamic acid (expressed as percentage change) between admission and 7 days after detoxification, Figure 2.

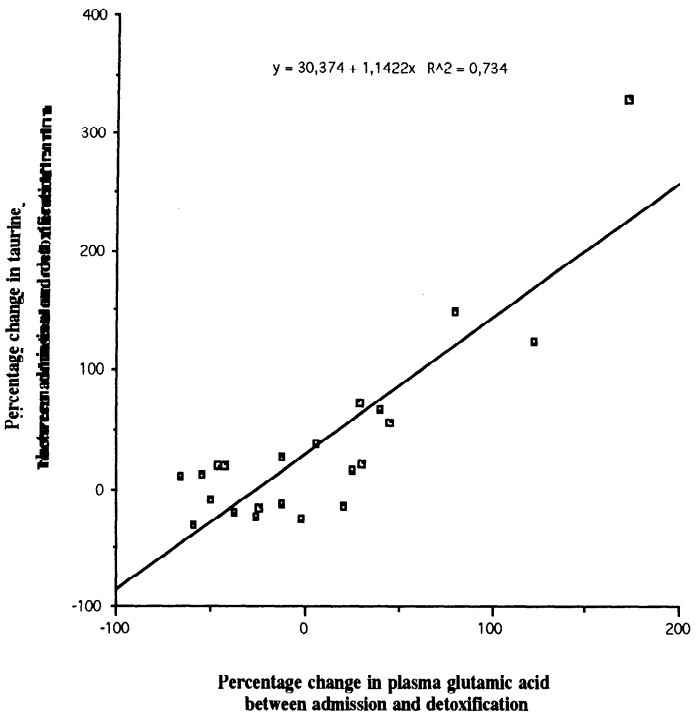

Figure 2. Correlation between the changes in glutamic acid and taurine (expressed as a percentage) in the plasma of chronic alcohol abusers before and after 7 days of medically assisted withdrawal.

The patients were further divided into two groups according to whether the concentration of glutamic acid had increased or decreased by greater than 10% between admission and after 7 days of detoxification. Table 2. In the 9 patients, where glutamic acid increased by an average of 57%, range 21-172%, there were significantly correlations, $r=0.89$, $P<0.001$, with the increases in plasma taurine content, (increasing by 87%). In addition, aspartic acid also increased between these two time points, by 30% in these patients, again significantly correlating with the changes in taurine content $r=0.76$ $P<0.01$. In the remaining group of patients, $n=10$, there were decreases in both glutamic and aspartic acid during the detoxification period by an average of 35%, range -13 to -66%, while the levels of taurine showed little

change. An inverse correlation between glutamate and glutamine was evident in the chronic alcohol abusers who ultimately showed an increase in glutamic acid after detoxification, r= -85.

The 9 patients who showed significant increases in both glutamic acid and taurine during the withdrawal period had significantly ($p<0.01$) lower scores on the withdrawal assessment scale (CIWA-Ar), (9.1 $\pm$ 3.9) compared with the remaining patients (12.5 $\pm$ 3.6). However no other significant differences in any of the other psychological evaluation, (SADQ or APQ) alcohol intakes, haematological or biochemical parameters were evident, Table 2.

Table 2. Clinical and biochemical parameters of two alcohol groups divided according to glutamate changes during medically assisted alcohol withdrawal

	Increase in glutamic acid	Decrease in glutamic acid
Age on admission	41 $\pm$ 11	38 $\pm$ 10
Years of dependence	21 $\pm$ 10	14 $\pm$ 11
Alcohol intake/day (g)	261$\pm$146	284$\pm$134
Questionnaires		
SADQ Score	38 $\pm$ 9	38 $\pm$ 9
CIWA-Ar Score	9 $\pm$ 4 **	13 $\pm$ 4
APQ	15$\pm$5	16$\pm$4
Total benzodiazepine dose	520 $\pm$ 145	590 $\pm$ 189
Biochemical and Haematological indices		
MCV	102$\pm$5	98.4$\pm$8
GGT	173$\pm$325	246$\pm$196
Uric acid	0.3$\pm$0.11	0.3$\pm$0.1
Albumin	43$\pm$5	46$\pm$3
Amino acids		
Plasma glutamic acid µmol/		
Before alcohol withdrawal	101 $\pm$ 61	196 $\pm$ 113
After alcohol withdrawal	166 $\pm$ 113	117 $\pm$ 4
Plasma taurine level, µmol/l		
Before alcohol withdrawal	61 $\pm$ 13	78 $\pm$ 10
After alcohol withdrawal	110 $\pm$ 50 **	75 $\pm$ 18

Significance ** = <0.01

DISCUSSION

Acute ethanol ingestion rapidly altered the plasma concentrations of taurine, alanine and glutamic acid, when the circulating ethanol concentration was approximately 100 mg%, taurine and alanine concentrations decreasing significantly while glutamic acid content increased. Whether this indicates changes in the uptake of these amino acids by the tissues is unknown. On admission for detoxification the

taurine levels in chronic abusers of alcohol, (with blood alcohol levels between 2-434 mg%), were similar to controls. However, once the blood ethanol concentrations had declined to zero, significant increases, were evident in approximately 50% of the patients, many above the reference range. This may indicate that ethanol alters the uptake of this sulphonated amino acid, but that there is adaptation by certain individuals to override this ethanol induced effect, which only becomes evident after detoxification. The only other parameter which was significantly different between these two groups of patients was a reduction in CIWA-Ar score; all other psychological evaluations, haematological and biochemical indices being similar.

The positive increase in both taurine and glutamic acid in 50% patients after detoxification may identify two groups of subjects with different responses to detoxification, although the clinical significance at this time remains unclear. Rats infused with morphine for 4 days showed no change in spinal dialysis amino acids, but when administered naloxone, initiating an antagonist-precipitated withdrawal, an immediate increase of both glutamate and taurine was evoked, by approximately 300% (20). The magnitude of these changes correlated with behavioural indices of withdrawal intensity. In contrast, in this present study an elevation of these two amino acids within the plasma in patients after alcohol withdrawal was associated with a lower anxiety score.

It is clear that there is an association between taurine and glutamate in a number of tissues; they are co-localised within the brain (21) particularly in dentritic outgrowth and synapse formations (22). Parallel changes in both glutamate and taurine occur in brain during hepatic encephalopathy (23), and in hypoxia and ischaemia (24). Other tissues, such as human saphenous vein for coronary artery bypass (25) and intestinal mucosa after trauma (26) also show comparable alterations in taurine and glutamate. Plasma levels appear to reflect brain concentrations, an increase in their concentration after acute exposure to bacterial endotoxin stress was paralleled in the brain. (27).

Circulating levels of taurine will reflect its absorption, its utilisation by the liver, particularly in the formation of bile salts, as well as muscle metabolism where taurine is present in high amounts and plays an important role in stabilising the sarcolemma (28). Sodium dependent taurine transporters have been identified in a variety of tissues e.g brain (29) kidney (30) intestinal cells CaCo-2 (31) which facilitate the uptake of taurine. It has been shown that taurine transporter activity is modulated by protein kinases (32), the latter being altered by ethanol administration; acute ethanol challenge inducing a rapid increase in the content of the phosphorylated form of cyclic AMP-response element-binding protein phosphorylation, peaking at 30 minutes, (33) which could explain the decrease in plasma taurine assayed after administration of 0.8 g ethanol/kg. Chronic ethanol exposure markedly attenuated the induction of this phosphorylation product (33), which was perhaps reflected by the lack of change in taurine levels observed on admission of the chronic abusers of alcohol for detoxification.

Both acute and chronic ethanol administration increased the circulating concentrations of glutamic acid. The explanation for the elevated glutamic acid after acute ethanol intake is unclear but may reflect in part, an effect of ethanol on the enzyme glutamine synthetase, inhibiting its metabolism to glutamine. Glutamine is synthesised from glutamate by amidation, glutamine synthetase catalysing the formation of glutamine in

a reaction in which NH_3 is the amino group donor and ATP is hydrolysed to ADP and P*i* via the intermediary of γ-glutamylphosphate. Inhibition of this pathway by ethanol may also be encountered in chronic alcohol abusers. An inverse relationship between glutamine and glutamic acid was identified in the plasma of chronic abusers of alcohol on admission who showed an increase in glutamic acid after detoxification, but neither the other group nor the two groups after 7 days of detoxification showed this significant inverse relationship. An earlier study of Tominaga (34) did not find any correlation between glutamate and glutamine in plasma of chronic alcohol abusers. The elevated glutamic acid level in chronic abusers of alcohol clearly is related to the extent of liver damage as described previously (34). However why it should further increase in 50% of the chronic abusers of alcohol during detoxification patients, (who were not consuming ethanol, as confirmed by random tests during this period), is unclear but may indicate changes in the uptake of glutamic acid into tissues by its transporters.

The biochemical explanation for the decrease in plasma alanine in our fasting normal subjects is associated with its key role in glucogenesis, as this α amino acid is readily deaminated in the liver. The ethanol induced redox shift in the liver might also increase the deamination of alanine to pyruvate.

Taurine homeostasis is clearly perturbed by both acute and ethanol administration, although the exact mechanisms involved await identification. Our present results identify that there is a differing responses by chronic abusers to alcohol withdrawal but how changes in certain circulating amino acids influence uptake of all amino acids across the blood brain barrier at this time is unknown. Clearly this zwitteronic sulphonated amino acid plays an important role during ethanol intoxication and withdrawal, such that clarification of its mode of action is of major importance.

We are grateful to Professor C. Cook for his help in the organisation of the clinical studies. Thanks are also due to the patients who were included in the study, to Dr François Rensberg, and to the laboratory staff both at the Bethlum Royal Hospital, Kent UK, and Dr M Vincent and Mrs Serneels, Cliniques St Luc, Brussels, Belgium for the analysis of the plasma amino acid. This work was supported by FRSM (1997-2000) and sponsored by LIPHA.

REFERENCES

1. Huxtable, R.J. (1992) Physiological Reviews 1, 101
2. Schaffer, S.W., Azuna, J. and Madura, J.D. (1995) Amino Acids 8, 231.
3. Smith, K.E., Borden, L.A. Wang, C.H., Hartig, P.R., Branchek, T.A. and Weinshank, R.L. (1992) Mol.Pharmacol. 42(4), 563.
4. Huxtable, R.J., Laird, H., Bonhaus, D. and Thies, A.C. (1982) Neurochem. Int. 4, 73.
5. Lleu, P.L. and Huxtable, R.J. (1992) Neurochem. Int.21, 109.
6. Gutierrez-Ruiz, M.C., Gomez, J.L., Souza, V. and Bucio, L. (1995) Cell. Biol. Toxicol. 11(2), 69.
7. Lieber, C.S., Robins, S.J. and Leo, M.A. (1994) Alcohol Clin. Exp.Res. 18(3), 592.
8. Dahchour, A. and De Witte, Ph. (1998) submitted.
9. Rossetti, Z.L. and Carboni, S. (1996) Eur. J. Pharmacol. 283, 177.
10. Dahchour, A., Quertemont, E. and De Witte, Ph. (1994) Alcohol & Alcohol. 29, 485.
11. Dahchour, A., Quertemont, E. and De Witte, Ph. (1996) Brain Res. 735, 9.
12. Tan, C.Y., Weaver, D.F. (1997) Seizure 6, 255.

13. Iida, S. and Hikichi, M. (1976) J. Study Alcohol 37, 19.
14. Besson, J. (1997) Schweiz. Med. Wochenschr. 127(38), 1574.
15. Majundar,S.K., Shaw, G.K., Thomson, A.D., Pratt, O. and Greenward, J. (1983) Med. Hypotheses. 12, 239.
16. Sullivan, J.T., Sykora, K. Scheiderman, J. Naranjo, C.A. and Sellers, E.M. (1989) Br. J. Addict. 84, 1353.
17. Drummond, D.C. (1990) Br. J. Addict. 85, 357.
18. Williams, B.T. and Drummond, D.C. (1994) Drug Alcohol Depend. 35, 239.
19. Stockwell, T., Murphy, D. and Hodgson, R. (1983) Br. J. Addict. 78, 145.
20. Jhamandas, K.H., Marsala, M., Ibuki, T. and Yaksh, T.L. (1996) J. Neurosci. 16(8), 2758.
21. Rassin, D.K., Sturman, J.A. and Gaull, G.E. (1982) Neurochem. Res. 7, 1107.
22. Magnusson, K.R. (1996) Adv. Exp. Med. Biol. 403, 435.
23. Buttherworth, R.F. (1996) Metab. Brain. Dis. 11(2), 165.
24. Saransoni, P. and Oja, S.S. (1998) Neurochem. Res. 23(4), 563.
25. Suleiman, M.S., Wallace, D. Birkett, S. and Angelini, G.D. (1995) Cardiovasc. Res. 30(5), 747.
26. Ahlman, B. Ljungqvist, O. Persson, B. Bindslev, L. and Wernerman, J. (1995) JPEN. J. Parenter. Enterol. Nutr. 19(4), 272.
27. al Shabanah, O.A., Mostafa, Y.H., Hassan, M.T., Khairaldin, A.A. and al-Sawaf, H. (1996) Res. Commun. Mol. Pathol. Pharmacol. 92(1), 95.
28. De Luca, A., Pierno, S. and Camerino, D.C. (1996) Eur. J. Pharmacol. 296(2), 215.
29. Chung, S.J., Ramanathan, V., Giacomini, K.M. and Brett, C.M. (1994) Biochem. Biophys. Acta 1193(1), 10.
30. Matsell, D.G., Bennett, T. Han, X., Budreau, M.M. and Chesney, R.W. (1997) Kidney. Int. 52(3), 748.
31. Satsu, H., Watanabe, H., Arai, S. and Shimizu, M. (1997) J. Biochem. Tokyo. 121(6),1082.
32. Loo, D.D., Hirsch, J.R., Sarker, H.K. and Wright, E.M. (1998) FEBS Lett. 392(3), 250.
33. Yang, X., Horn, K., Baraban, J.M. and Wand, G.S. (1998) J. Neurochem. 70(1), 224.
34. Tominaga, T., Suzuki, H., Mizuno, M., Kuono, M., Suzuki, M., Kato, Y., Sato, A., Okabe, K. and Miyashita, M. (1993) Alcohol Alcohol suppl. 1A, 103.